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ENZYMIC HYDROLYSIS OF POTATOES[1]

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Introduction

During recent years, cull and surplus potatoes have been used in enormous quantities for alcoholic fermentation and in small amounts for other fermentations. Industry has needed more information on the use of different types of amylases in converting the starch of potatoes to sugars prior to fermentation. Knowledge pertaining to the hydrolysis of potatoes is also necessary to further their use in applications other than fermentations.

This study on enzymic hydrolysis of potatoes was instituted to obtain data on better methods of producing potato hydrolyzates for fermentations and for such uses as adhesives, binders, sizes, thickeners, and glues. Commercial amylase preparations are herein compared in terms of their ability to convert starch into dextrins and sugars. An amylase that gives predominantly dextrins and relatively little sugars would be of value when

[1] Report of a study made under the Research and Marketing Act of 1946.
[2] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, United States Department of Agriculture.

the starch of potatoes is to be converted into a viscous paste of high cohesive and adhesive strengths for use as an adhesive, for example. On the other hand, an amylase that converts starch primarily to sugars is required when potatoes are used as raw material for fermentations or for preparation of sirups.

Procedures

Cooking. — Washed potatoes were ground in a hammer mill, mixed with 0.1 per cent RHozyme DX[3] liquefying enzyme (based on the weight of starch), and autoclaved. Since the potatoes contained 13 per cent starch on the average, the amount of liquefying enzyme added was ordinarily about 0.013 gram per 100 grams fresh potatoes. Five thousand grams of ground potatoes were used per batch in a 2-gallon glass-lined autoclave equipped with a stirrer. About 1 hour was required to bring the potatoes to 130° C. During this period, the temperature was maintained at 40° to 60° for at least 15 minutes to give the liquefying enzyme a chance to thin the starch. After this, the enzyme was destroyed by raising the temperature to 130°-135°, and holding it for ½ hour. The sole purpose of using the liquefying enzyme was to render the cooked material fluid enough to handle. It was determined that use of this low concentration of enzyme for 15 minutes produced no measurable amount of sugar.

Hydrolysis. — After the autoclaving, the cooked potatoes were blown out of the pressure vessel. Portions of 1000 grams were placed in containers held in a constant temperature bath and provided with efficient agitation. The naturally occurring pH of the potatoes (about 5.5) was not altered except when pH was studied as a variable. As soon as the temperature of the cooked potatoes equalled that of the bath, the enzyme (usually 1 per cent by weight based on the amount of starch or what amounted to about 1.3 grams per 1000-gram portion of potatoes) was added in a water solution or suspension. Although this amount of enzyme is greater than that ordinarily recommended by manufacturers, it was not an excessive concentration. Addition of the enzyme was taken as zero time in the hydrolysis. Samples were removed after ½, 1½, 2½ and 4 hours. The 100-ml. samples were placed in a 500-ml. Erlenmeyer flask immersed in a 90° C. bath; enzymic activity was stopped in a few minutes at this temperature. The samples were then ready for analysis.

Preparation of Sirups. In the preparation of sirups, hydrolysis was similar to the rest except that higher enzyme concentration was used to assure high conversion to sugars in 1 hour at 55° C. For example, 15 per cent barley malt (based on the weight of starch) was used in one case. The hydrolyzate was filtered and given one activated carbon treatment, after which it was passed through a cation exchange resin column (Amberlite IR-120)

[3] Products mentioned in this paper are not recommended or endorsed by the U. S. Department of Agriculture over other similar products not mentioned.

followed by passage through an anion exchange resin (Amberlite IRA-400) to remove the positive and negative ions of the amino acids, other nitrogen compounds, inorganic salts, and other compounds of the potato. Permutit DR, a decolorizing resin, was as efficient as activated carbon and much more convenient to use[8]. The hydrolyzate was then given another carbon treatment and concentrated under reduced pressure at 35° to 45°.

Analytical Determinations.

Total Solids. The 15 to 20 gram sample was dried for 2 hours at 65° C. in a mechanical convection oven, followed by drying to constant weight in a vacuum oven at 70°.

Starch. The method of Steiner and Guthrie (7) and the shorter method of Clendenning (1), which gave essentially the same result on identical samples, were used.

Dextrose and Maltose. The hydrolyzate was prepared for the sugar determinations by use of the A.O.A.C. method (3).

The determinations were made by the Zerban and Sattler modification of the Steinhoff method (8), in which dextrose plus maltose, are determined jointly, dextrose is determined separately, and maltose is calculated by difference.

Total Sugars. After the crude hydrolyzate was treated by the previously referred to A.O.A.C. method to obtain the extract, the solution was hydrolyzed with one-tenth of its volume of 10 per cent hydrochloric acid (specific gravity 1.125) and sugars determined as dextrose. (4)

Total Soluble Carbohydrates. The sample (15 g.) was extracted by shaking with 500 ml. distilled water at 25° - 30° C. and letting the mixture stand for 1 hour. An aliquot part of the filtered extract was then hydrolyzed as in the total sugars determination. Sugars were determined as dextrose.

Higher Sugars. Oligosaccharides other than maltose were calculated by subtracting dextrose plus maltose from total sugars. It is assumed that the reducing power of these higher sugars can be neglected for all practical purposes.

Data and Discussion

Amylases Used. Amylases, like other enzymes, are proteins. They exist in crystalline form when pure, and have high molecular weights. Each amylase has its optimum temperature, pH, and environment. Commercial amylase preparations high in alpha-amylase liquefy starch, producing dextrins quickly. Alpha-amylase is a versatile amylase, however, that not only liquefies starch in its primary phase but also produces sugars by its later action. Beta-amylase produces maltose primarily and does not liquefy starch rapidly.

Twelve commercial preparations, sprouted barley (barley malt), and a

recently developed fungal amylase were evaluated. There was considerable variation in the potency of the preparations. This was expected, since some commercial amylases are extracts of the soluble constituents, whereas others, like sprouted barley, contain a preponderance of non-active material. Some commercial amylases also contain inert diluents.

Table 1 gives the origin and potency of the various preparations in terms of alpha- and beta-amylase units. Alpha-amylase values were obtained by the method of Olson, Evans, and Dickson. (5) The only change made in their procedure was the determination of activity at 30° C., as previously practiced by Sandstedt, Kneen, and Blish (6), instead of at 20°.

This method was originally developed for use with barley malt. Although it has been employed to assay amylases of other origins, the method has limitations with amylases other than malt.

Beta-amylase activity was determined by the method of Kneen and Sandstedt. (2)

TABLE 1.—*Potency of Commercial Amylases*

Enzyme	Source	Alpha-Amylase Units*	Beta-Amylase Units†
Group 1, saccharifying:			
Sprouted barley	Plant	25	11.4
Malt sirup (Malt Diastase Co.)	"	100	Not determined
Pancreatin (Takamine Lab., Inc.)	Animal	375	‡
Malt diastase " " "	Plant	400	19
Enzyme MB (Wallerstein Labs.)	Fungus	2,400	0
Clarase (Takamine Lab., Inc.)	"	4,957	0
RHozyme S (Rohm & Haas Co.)	Microorganism	6,810	0
Dextrinase (Takamine Lab., Inc.)	Fungus	7,525	0
Enzyme BS " " "	§	9,240	67
Fungal diastase (Paul-Lewis Labs.)	Fungus	15,967	0
Group 2, liquefying:			
RHozyme DX (Rohm & Haas Co.)	Fungus	194	1.2
Rapidase W3F (Wallerstein Labs.)	Bacterial	333	6.8
Vanzyme 31 (R. T. Vanderbilt Co.)		1,695	13.3

* Grams soluble starch which in the presence of an excess of beta-amylase are dextrinized by 1 gram enzyme in 1 hour at 30° C.
† Grams soluble starch converted to maltose by the beta-amylase of 1 gram enzyme in 1 hour at 30° C.
‡ Result considered doubtful.
§ Source not disclosed by manufacturer.

As exemplified by the typical cases shown in figures 1 and 2, the amylases used in this study fell into two groups: (1) Saccharifying enzymes that give a high sugar concentration, principally maltose as a rule, early in the hydrolysis, and (2) liquefying enzymes that quickly convert starch to dextrins but require considerable conversion time for production of appreciable sugars. For this reason, the amylases are separated into two groups both in table 1 and in the discussion of the hydrolyses.

The results obtained in the enzymic hydrolysis of potatoes did not correlate unusually well with the alpha- and beta-amylase potencies as determined by standard procedures. Table 1 shows that, as expected because of the nature of their hydrolyses, two of the three amylases of Group 2 had low beta-amylase potencies and reasonably high alpha-amylase potencies. Enzymic preparations high in alpha-amylase, however, produced

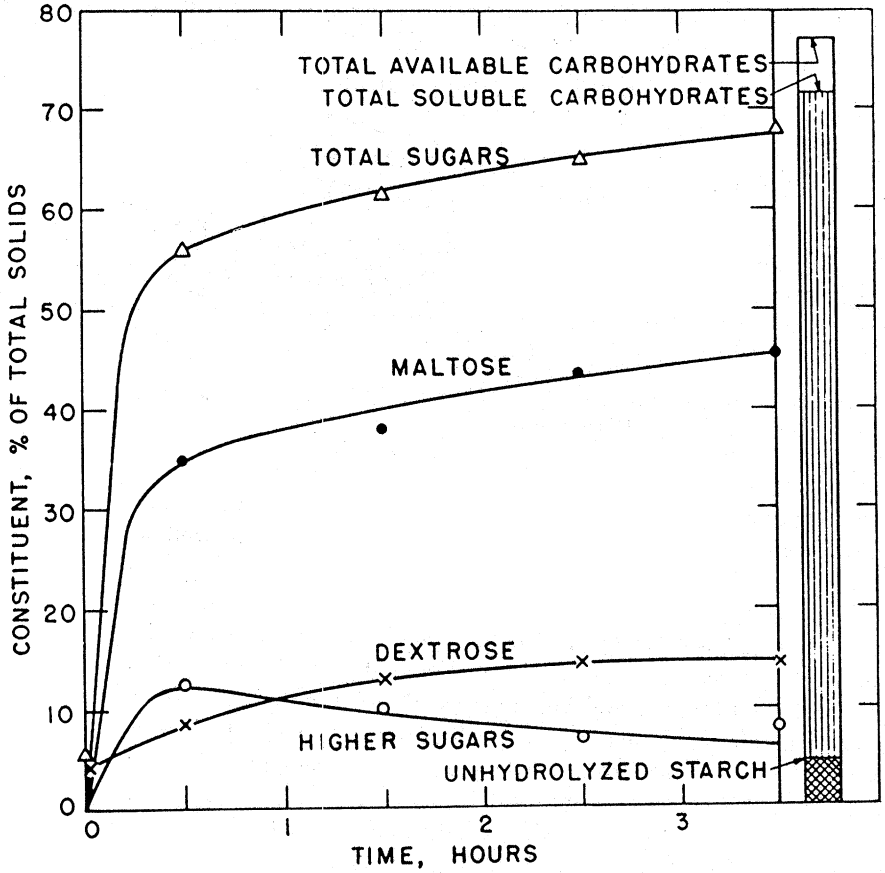

Fig.. 1. — Hydrolysis of potatoes with commercial saccharifying-type amylase (RHozyme S)

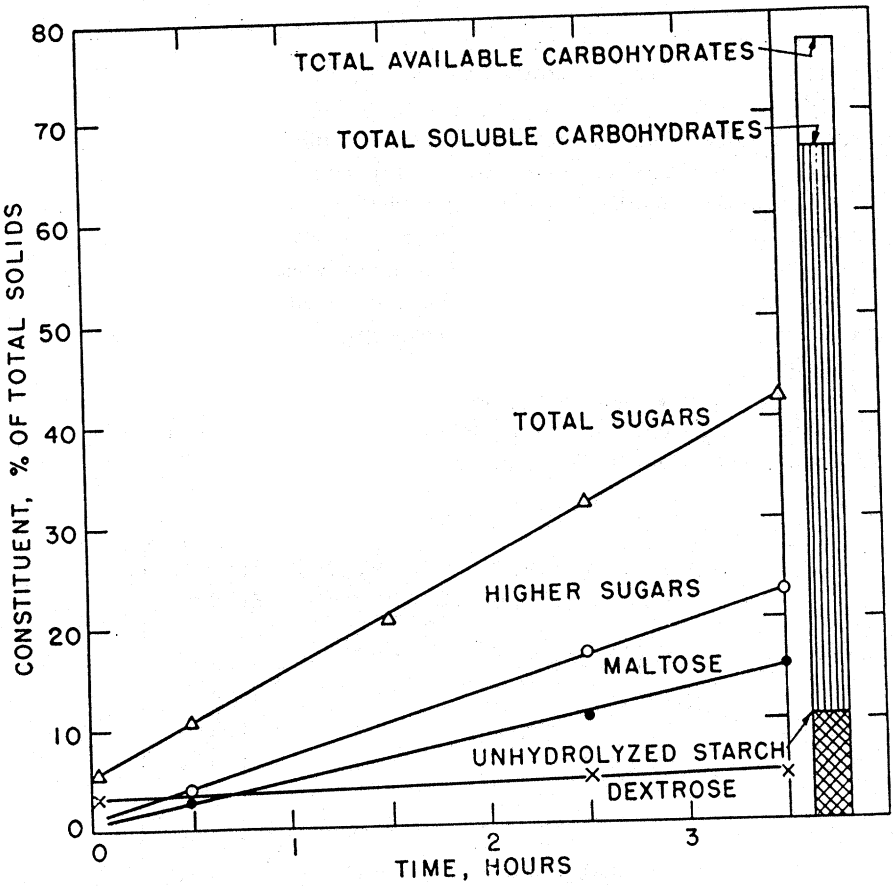

Fig. 2. — Hydrolysis of potatoes with commercial liquefying-type amylase (RHozyme DX)

rapid saccharification, even when beta-amylase was low or *nil*. This bears out the fact that alpha-amylase is a fundamental amylase and, when present in sufficient potency, will bring about saccharification as well as liquefaction.

Dextrinogenic activity is a function principally of alpha-amylase, but alpha-and beta-amylase overlap in their saccharogenic activity. Some of the commercial amylases used undoubtedly contained maltase and other carbohydrases in addition to alpha- and beta-amylase, but it was not an object of this study to characterize them in detail. No attempt is made, therefore, to explain why Group 1 and Group 2 commercial amylases gave different types of hydrolysis.

Hydrolyses by Group 1 Amylases. All the amylases of Group 1 rapidly produced sugars, of which maltose predominated. Since similar results

TABLE 2.—*Hydrolysis of potatoes by various commercial saccharifying amylases. Carbohydrate composition of hydrolyzate after 30 minutes hydrolysis 1 per cent enzyme; 55° C.*

Enzyme	Dextrins	Total Sugars	Higher Sugars	Maltose	Dextrose
Sprouted barley*	13	54	6	43	5
Malt sirup	28.5	41.5	2.5	33.5	5.5
Pancreatin	17.5	55	7	39.5	8.5
Enzyme MB	23.5	45	16	22.5	6.5
Clarase	13.5	52.5	19.5	27.5	5.5
RHozyme S	15.5	56	12.5	35	8.5
Dextrinase	14.5	61	11	39	11
Enzyme BS	21.	50.5	24	20	6.5
Fungal diastase	9	65.5	13.5	45.5	6.5

* 15 per cent by weight, based on starch weight.

were obtained for all 10 amylases of this type, one of the group (RHozyme S) was selected as typical and its curves illustrating rate of sugar formation are presented. Figure 1 shows the carbohydrate composition of the hydrolyzate during 3½ hours of hydrolysis. Table 2 gives compositions of the hydrolyzates obtained with the various saccharifying amylases.

These enzymes produced total sugars at a high rate initially and then at a slower rate after one-half hour or so. No attempt was made to investigate the kinetics of the hydrolyses, since the enzyme mixtures in commercial amylase preparations would make this too complicated. The "higher sugars" (unknown oligosaccharides) produced in the hydrolysis, although present in smaller amount than maltose, were generally found in greater concentration than dextrose at the ½-hour sampling time. They usually decreased during the following hour, as dextrose increased. The values of the higher sugars typically assumed a low level after 3 or 4 hours' hydrolysis, at which time the sugars were nearly all maltose and dextrose.

The total soluble carbohydrates values were always a little less than the total available carbohydrates (starch plus total sugars) values. A small amount of unhydrolyzed starch was invariably left at the end of a conversion and remained unextracted during the determination of total soluble carbohydrates. The amount of unhydrolyzed starch found by direct determination was frequently equal to the difference between total available and total soluble carbohydrates, within experimental error.

Hydrolyses by Group 2 Amylases. RHozyme DX was the slowest sugar

TABLE 3.—*Hydrolysis of potatoes by commercial liquefying amylases. Carbohydrate composition of hydrolyzate after 30 minutes hydrolysis 1 per cent enzyme; 55° C.*

Enzyme	Dextrins	Total Sugars	Higher Sugars	Maltose	Dextrose
RHozyme DX	56	10.5	4	3.5	3
Rapidase W3F	40	23.5	13.5	5.5	4.5
Vanzyme 31*	26	48.5	24.5	19	5

* This enzyme is sold by the manufacturer as a starch liquefying agent for production of adhesives and sizes. Conditions used in our hydrolysis, which resulted in high sugar formation, were considerably different from the enzyme concentration, pH, and temperature recommended by the manufacturer.

producer of all the amylases studied. Figure 2 gives the rates of formation of sugars in the hydrolysis of potatoes by this enzyme. The per cent sugar-time relationships are nearly linear. Table 3 shows the composition of the hydrolyzates obtained with three liquefying amylases.

Rapidase W3F also produced sugar slowly, although it had high dextrinizing power. Vanzyme 31 was used at 1 per cent concentration based on the starch, at pH 5.5, and at 55° C. in order to compare its activity with other commercial amylases under the same conditions. It produced much sugar, however, likely because the recommended conditions for using it as a liquefying amylase are far different from those we employed. The manufacturer's recommendations call for a lower enzyme concentration, a higher pH, and a considerably higher temperature. The amount of higher sugars formed in the hydrolysis by each of these three amylases was roughly equal to the sum of maltose and dextrose. The liquefying enzymes differed in action from Group 1 amylases in that the concentration of higher sugars increased continuously during the 4-hour conversion. Dextrose values remained almost constant at low levels.

Action of a Commercial Hemicellulase. A sample of hemicellulase obtained from the Paul-Lewis Laboratories saccharified potatoes well. In one experiment, the potatoes originally contained 67.5 per cent starch and 4.8 per cent total sugars on the dry basis. After 4 hours' hydrolysis at 55° with 1 per cent added hemicellulase (based on weight of starch), the hydrolyzate consisted of the following (per cent, dry basis): Total soluble carbohydrates (expressed as dextrose equivalent after acid hydrolysis of solubles) 66.5; dextrose 9.4; maltose 19.9; higher sugars 13.4; dextrins 21.4.

A mixture of 1 per cent hemicellulase and 1 per cent amylase (starch weight basis) added to cooked, ground potatoes gave a significantly higher soluble carbohydrates value than that accounted for by adding the starch and total sugars values. Clearly the hemicellulase attacked higher carbo-

hydrates that are untouched by amylases. The hydrolyzate was not clear, however. Even if all the hemicellulose were converted to soluble form, the corky skin tissue (and perhaps some cellulose) would remain undissolved.

Effect of the Non-Amylaceous Constituents of the Potato on Enzymic Hydrolysis of Starch.

To determine how hydrolysis of the starch in potato differs from hydrolysis of pure potato starch (in distilled water), several amylases were used in comparative conversions of both substrates. Since amylases are commonly affected by the presence of various substances in the medium, it was expected that the nitrogen compounds, salts, and acids of the potato would alter the results. The pH was about 5.5, and the starch concentration was the same in both the aqueous starch and potato media. Data for some of the Group 1 and Group 2 amylases are given in table 4. To conserve space, data are given for 4 hours' conversion only and for only two enzymes in each classification. The amount of starch solubilized was greater when pure starch was used. Group 1 amylases converted a higher proportion of pure starch than starch in potato to dextrose. The saccharifying amylases gave about the same conversion to maltose for the two substrates. Group 2

TABLE 4.—*Comparative hydrolyses of potatoes and pure starch by commercial amylases 1 per cent amylase; 55° C.*

Enzyme	Substrate	Per cent Starch Originally Present*	Per cent Starch Converted after 4 Hours to — Total Soluble Carbohydrates	Maltose	Dextrose	Higher Sugars
Group 1 Saccharifying Amylases						
Enzyme MB	Potato	64.1	85.2	59.7	8.8	6.0
	Starch	98.5	94.1	53.4	23.3	—†
RHozyme S	Potato	64.0	92.8	65.1	15.6	11.2
	Starch	98.5	100.9	61.7	19.8	—
Group 2 Liquefying Amylases						
RHozyme DX	Potato	69.2	79.6	22.8	1.6	33.8
	Starch	98.5	90.4	33.9	2.6	—
Rapidase W3F	Potato	61.7	81.7	26.3	0	32.2
	Starch	98.5	91.0	31.8	0	—

* Expressed on dry basis.
† Not determined.

amylases differed from Group 1 in that they produced little or no dextrose when acting on either substrate; they converted a higher amount of pure starch than starch in potato to maltose.

Effects of pH, Temperature, and Enzyme Concentration

We have found that little or nothing is gained in enzyme activity by adjusting a potato slurry from its natural pH (about 5.5) to the pH recommended by the amylase manufacturer for starch hydrolysis. Not only rate of hydrolysis but also composition of the hydrolyzate is nearly the same, whether or not the conversion takes place at the pH of the potato or at the recommended pH; sometimes addition of sodium hydroxide or hydrochloric acid has a detrimental effect. Owing to the buffering action of the salts and nitrogen compounds of potato juice, a relatively large amount of either acid or alkali is required for changing the natural pH. The optimum pH of the various amylases used, as recommended by the manufacturers, ranged from 4 to 7.5.

Most commercial amylases, except those of bacterial and pancreatic origins, have optimum temperatures near 55° C., the temperature at which comparisons were made. Only minor changes, therefore, have usually resulted when hydrolysis was carried out at the recommended temperature for a preparation instead of at 55°. A commercial fungal diastase (product of the Paul-Lewis Laboratories), having a recommended optimum temperature of 45°, gave virtually the same results at 55°. Even a wide variation in temperature usually made little difference in the hydrolysis. Another fungal diastase (Dextrinase), with optimum temperature 40°, gave similar results at 65°.

The composition of a potato hydrolyzate varied within wide limits, however, with changes in enzyme concentration. Higher enzyme concentration does not simply make the hydrolysis proceed faster in a pattern similar to that obtained at low amylase concentration. For example, dextrinase gave a family of smooth curves for rate of dextrose formation with increasing amounts of enzyme (0.5 to 2.5 per cent). The maltose concentration increased continuously with time when 0.5 per cent of this amylase was present, but passed through maxima with 1.5 and 2.5 per cent enzyme. Maltose concentration, in the latter case, was reduced during the later stages of the hydrolysis, as it was split to dextrose.

Sirups

Glucose sirup has been prepared commercially from potato starch by acid hydrolysis in a manner similar to production of corn sirup. One or two companies that have made sirup by enzymic hydrolysis of starch in the presence of other constituents of the potato, however, have met with difficulties in obtaining a clear, light-colored product. As a part of our

study on enzymic hydrolysis of potatoes, sirups were produced directly from potatoes in the hope that either an improved process or a product of unique flavor could be developed.

Using the general methods described in this paper, we employed a commercial fungal amylase (Paul-Lewis Laboratories) to convert ground, cooked potatoes to a hydrolyzate of high sugar content. A sweet, amber-colored sirup of pleasing taste was produced.

Bland, virtually colorless sirups of high clarity and sweetness were produced by either barley malt or commercial amylases. The potato hydrolyzates were concentrated after they had been treated with activated carbon and ion exchange resins. Analyses showed that the nitrogen contents of these sirups were low. It is likely that the organic acids and inorganic salts contents were also low after the ion exchange treatments. As far as we are aware, this is the first preparation of a white, bland sirup by enzymic hydrolysis of potatoes. The flavor of potato sirup is similar to that of ordinary corn sirup.

Several attempts were made to prepare an acceptable potato sirup by using amylase from *Aspergillus niger* NRRL 330[4], cultured *in situ*[5], for the starch conversion. The sirups were of poor flavor, perhaps because of acid produced by this strain of *A. niger*. Probably other types of fungal amylase would be more suitable for producing sirups in this way.

Summary and Conclusions

A number of commercial amylases have given rapid conversion of starch in potato to sugars. Most of the amylases produced nearly their maximum amount of sugar in ½ hour at 55° C. with 1 per cent enzyme, based on weight of the starch.

Maltose was the principal sugar produced by all amylases. Unknown oligosaccharides were produced in the hydrolyses, generally in greater amount than dextrose in the early phase. These oligosaccharides, which may well be unfermentable in several of the most common types of fermentations, can be reduced to an insignificant amount either by carrying out the conversion for 3 or 4 hours or by using a higher concentration of amylase.

There was not much correlation between the alpha- and beta-amylase potencies of the commercial amylases and their performance in hydrolyzing potatoes under the conditions used. As expected, the slow sugar-forming amylases were relatively low in beta-amylase potency, but preparations

[4] This strain of *A. niger* was found by the Northern Regional Research Laboratory, Peoria, Ill., to be a good producer of amylases.

[5] The culture of *A. niger* NRRL 330 on potatoes and the conversion of starch to sugars were carried out simultaneously by E. A. Weaver of the Eastern Regional Research Laboratory.

having little or no beta-amylase but powerful alpha-amylase activity produced sugars rapidly.

The amylases used converted more pure starch than starch in potato to sugars, under the same conditions.

It apparently is unwise to change the naturally occurring pH of potatoes to be enzymically hydrolyzed. Results obtained with pH adjusted (by sodium hydroxide or hydrochloric acid) to the optimum recommended by amylase manufacturers were no better, and sometimes worse, than the corresponding control.

Recommended temperatures for pure starch conversions by commercial amylases did not apply in a critical manner to the potato hydrolyses.

The composition of potato hydrolyzates varied widely with changes in the enzyme concentration.

Virtually colorless sirups of high degree of sweetness and pleasant taste have been prepared from purified potato hydrolyzates.

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